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Preliminary results on first MetaboHUB NMR ring test

Acquisition and annotation of the “NIST standard human plasma” (SRM1950)

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the French national infrastructure of metabolomics and fluxomics provides tools and services to academic research teams and industrial partners in the fields of health, nutrition, agriculture, environment and biotechnology.



4 NMR SITES:

- Bordeaux Metabolome Facility (PMB), AVANCE III 500 MHz with a BBI RT-probe (5 mm NMR tube)
- Clermont-Ferrand PFEM, AVANCE III 500 MHz with a 60 μ L flow-cell probe (MetabolicProfiler)
- Toulouse MetaToul- Réseaux Métaboliques AVANCE III 800 MHz with a cryo-probe (3 mm NMR tube)
- Toulouse MetaToul-AXIOM AVANCE III 600 MHz with a cryo-probe (5 mm NMR tube)

A STANDARD REFERENCE MATERIAL AND NMR ANNOTATION KNOW-HOW TO SHARE

The standard reference material of human plasma (SRM1950) of the National Institute of Standards and Technology (NIST) was chosen to evaluate the 4 NMR sites of MetaboHUB in their way to (i) acquire 1D ^1H -NMR and 2D ^1H - ^{13}C -NMR spectra and annotate them using in-house NMR databases, PeakForest, HMDB, MMCD or BMRB databases, and (ii) share know-how between sites.

NIST STANDARD REFERENCE MATERIAL OF HUMAN PLASMA (SRM1950) PREPARATION

MetaToul-AXIOM was in charge of the preparation and distribution of the plasma NIST samples. 500 μ L of NIST plasma sample were treated as follow:

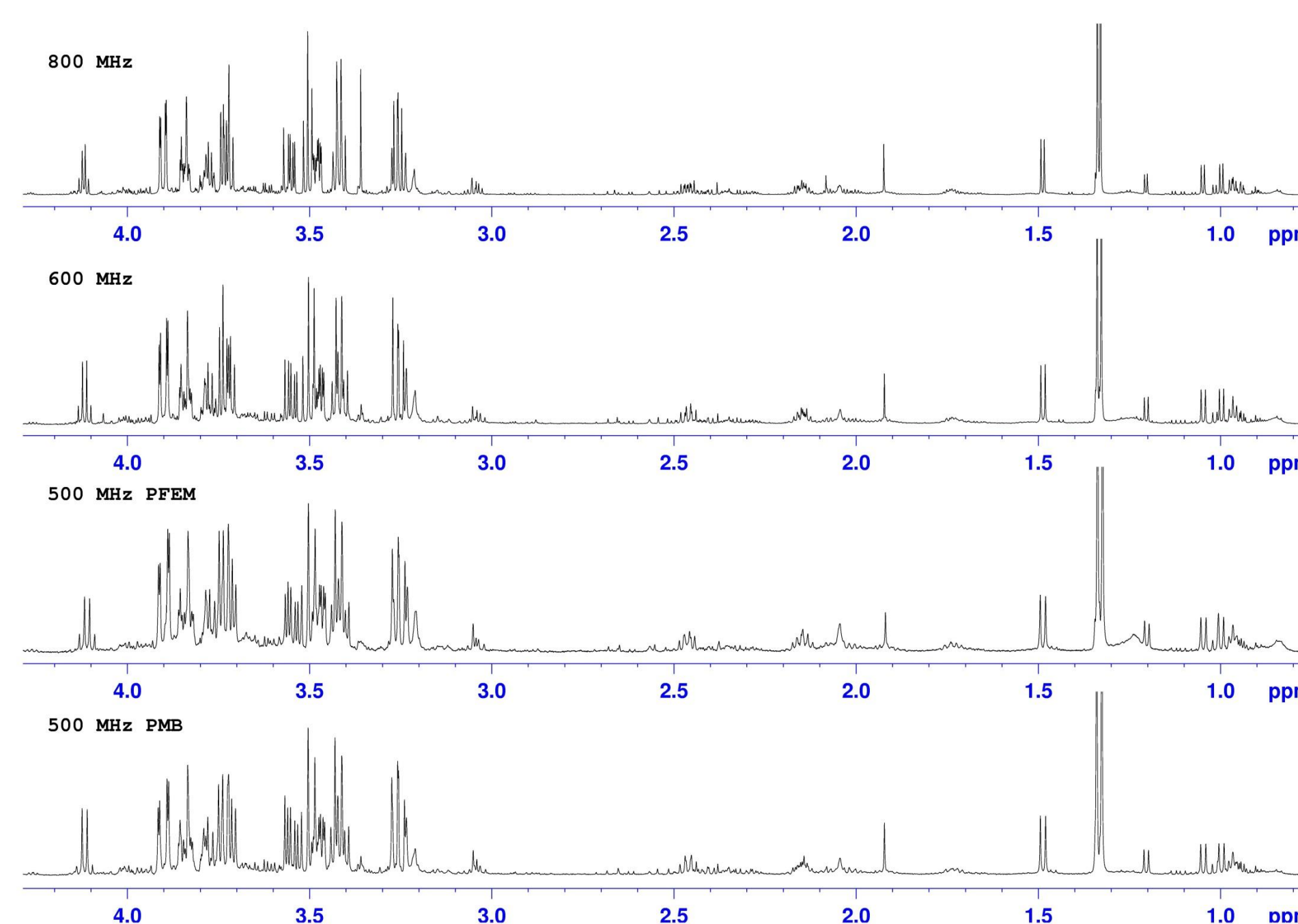
- Precipitation of proteins with 1 mL cold methanol, 30min at -20°C , centrifugation.
- Evaporation of (4 X 300 μ L) supernatant under a nitrogen flux.
- Shipment on dry ice to PFEM.
- Dilution of the resulting deproteinized plasma with a deuterated phosphate buffer (200 mM, pH 7; TSP) solution to a final volume of 600 μ L for 5 mm NMR tubes and 200 μ L for 3 mm NMR.
- Shipment on dry ice to PMB.

PRELIMINARY RESULTS

Progress of NMR acquisitions/annotation at 4 sites

Sites	NMR Acquisition			NMR Annotation		
	1D ^1H (cpmgpr1d)	2D ^1H - ^1H	2D ^1H - ^{13}C	Identification	Validation	Cross-validation
800 MHz	✓	✓ COSY TOSY	✓ HSQC HMBC	✓ Automatic (Chenomx)	In progress	In progress
600 MHz	✓	✓ COSY	✓ HSQC	✓ Manual	✓ Home-made database; PeakForest; HMDB	
500 MHz (Metabolic Profiler)	✓	✓ COSY TOSY JRES	✓ HSQC HMBC	✓ Manual	In progress	
500 MHz	✓	✓ COSY	✓ HSQC	✓ Manual	✓ Home-made database; HMDB; BMRB	

Comparison of first NMR acquisitions (cpmgpr1d) at 4 sites

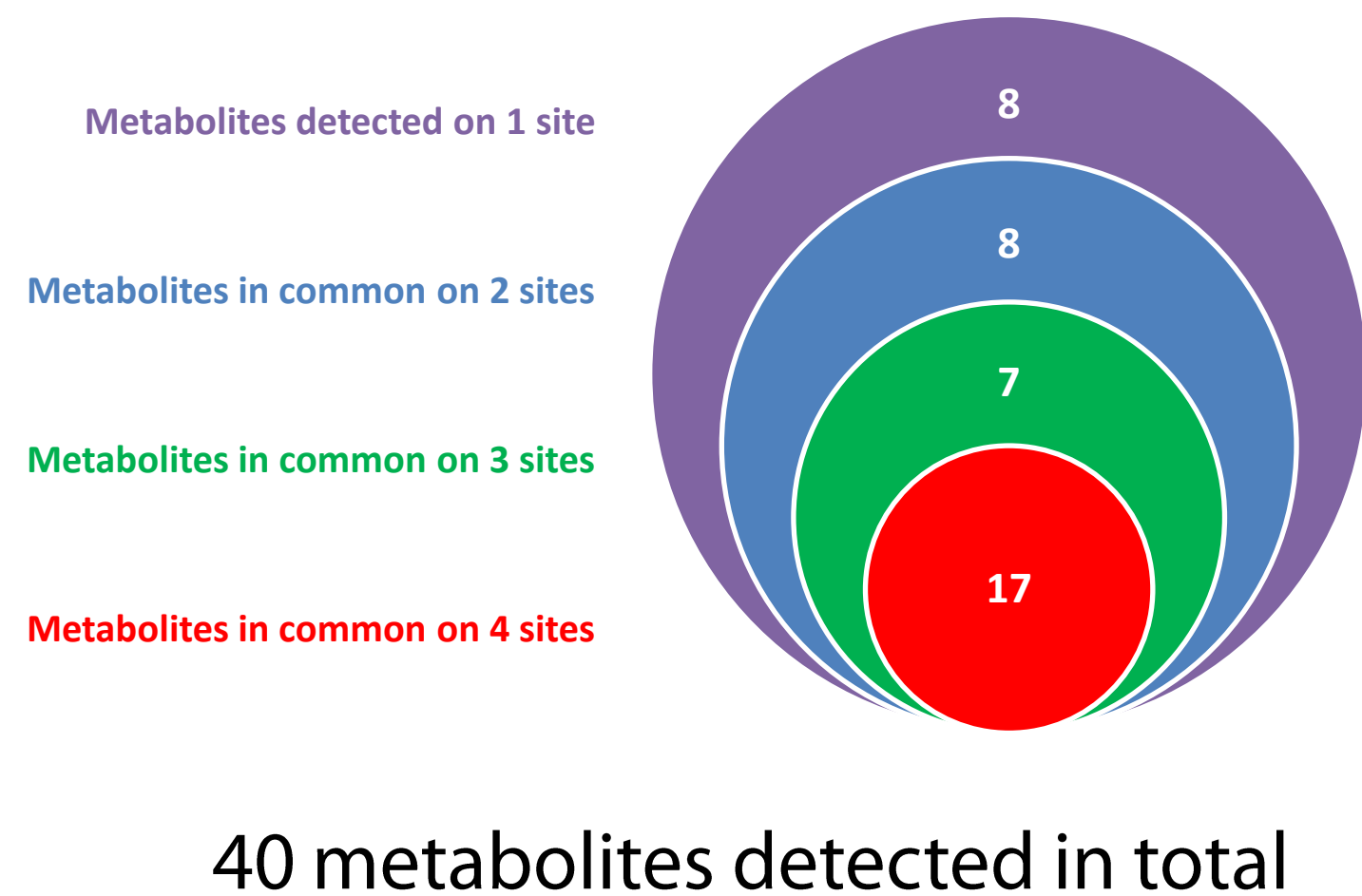


Example of Annotation table

Metabolite	ν (^1H) (ppm)	H's	Type (s, d, dd, t, q, m)	ν (^{13}C) (ppm) (HMDB)	COSY correlation	atoms	Home-made database	PeakForest database	HMDB database	Other database
3-hydroxybutyrate	1.2	3	D	24.7	4.15	4	checked and validated	checked and validated	checked and validated	not checked
3-hydroxybutyrate	2.31	1	Dd	49.4	4.15	2	checked and validated	checked and validated	checked and validated	not checked
3-hydroxybutyrate	2.41	1	dd	49.4	4.15	2	checked and validated	checked and validated	checked and validated	not checked
3-hydroxybutyrate	4.15	1	m	68.5	1.2; 2.31; 2.41	3	checked and validated	checked and validated	checked and validated	not checked
Acetate	1.92	3	s	26.16		2	checked and validated	checked and validated	checked and validated	not checked
Alanine	1.485	3	d	19.04	3.79	3	checked and validated	checked and validated	checked and validated	not checked
Alanine	3.79	1	q	53.26	1.485	2	checked and validated	checked and validated	checked and validated	not checked
Lactate	1.33	3	d	22.95	4.11	2	checked and validated	checked and validated	checked and validated	not checked
Lactate	4.11	1	q	71.15	1.33	3	checked and validated	checked and validated	checked and validated	not checked

A 2-sites exchange generated the construction of a template to store and report the chemical shifts used to attribute the NMR signals to metabolites and the databases used for validation

Overview of metabolite overlapping



First comparisons

Comparison of the number of identified metabolites by NMR between		
MetaboHUB	Official NIST website	Simón-Manso <i>et al.</i> , Anal. Chem. 2013
in common with	23 among 23	27 among 39
different with	17 identified by MetaboHUB and not by NIST	11 identified by MetaboHUB and not by Simón-Manso <i>et al.</i> 12 identified by Simón-Manso <i>et al.</i> , and not by MetaboHUB

CONCLUSION AND PERSPECTIVES

40 metabolites detected in total. The cross validation is still in progress between sites.

Difference between our results and those published in 2013 on “native” Plasma (Simón-Manso *et al.*; 2013) and the official NIST website are still under investigation.

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